COMMENTS ON

DRAFT NTP-CERHR EXPERT PANEL REPORT

ON

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF METHANOL

PREPARED BY KENNETH MCMARTIN, PH.D.
LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER - SHREVEPORT
DEPARTMENT OF PHARMACOLOGY
SHREVEPORT, LA 71130-3932

KMCMAR@MAIL.SH.LSUHSC.EDU
318-675-7871

SEPTEMBER 4, 2001

The Panel presents results from methanol ingestion and methanol inhalation studies from several different species, with the former in doses of mg/kg and the latter in concentrations of ppm over varying durations.

- ï In order to compare the total doses among the various studies, the Panel should convert the inhalation doses to an equivalent mg/kg dose, using standard formulae, and present these dosages for side-by-side comparisons. This would allow the Panel and others to more easily compare the results from the various studies among numerous species and to more readily draw relevant conclusions regarding assessments of risk.

The studies of oral ingestion of aspartame in humans offer insights into the metabolism of methanol after oral ingestion of non-lethal doses of methanol. The studies by Stegink et al (15,26) showed that extremely high doses of aspartame in humans, equivalent to methanol intake of up to 20 mg/kg, produce dose-related increases in blood methanol concentrations (up to 26 mg/L), with no increase in blood formate concentrations.

- ï The Panel noted that the detection limit for methanol in these studies was significantly higher than in more recent studies, thus limiting the time course of blood methanol levels at the lowest apparent methanol dose (3.4 mg/kg). However, even with the higher detection limits, Stegink et al. (15,26) were able to quantitate blood methanol levels in human subjects over significant periods of time at three higher dose levels. Hence, at the higher detection limits of Stegink et al., toxicologically meaningful levels of blood methanol would be detected.
- ï Although the Panel cited studies by Davoli et al. (27), they did not offer an evaluation of this study in terms of strengths, weaknesses or utility. The strengths of this study include a more sensitive method for methanol, GCMS, and a comparison of blood methanol accumulation after aspartame doses of 34 mg/kg in rats (same as the lowest dose in the Stegink studies) and of 6-8.7 mg/kg in humans. These doses increased blood methanol concentrations from 1.3 to 3.1 mg/L in rats and from 1.7 to 2.5 mg/L in humans. These increments were less than the detection limit of the Stegink studies and are in the range of the methanol levels due to normal variations in diet. Hence, consumption of very high amounts of aspartame will increase blood methanol to measurable, but non-toxicologically significant levels, without increasing blood formate concentrations.

The Panel can conclude from current data that methanol is developmentally toxic in rats and mice that are exposed to very high doses of methanol via inhalation or ingestion. The question becomes, how relevant are these data to human exposure? The Panel must seriously consider whether methanol itself or its metabolite formate is more likely to produce developmental effects in humans, particularly considering that humans are exposed to much lower doses in their diets or environment. A corollary to this concern would be to consider the appropriateness of rodents as animal models for the effects at environmental exposure levels of humans to methanol.

- ï The Risk Assessment or Summary Conclusions in the Final Report must consider the relevance of the critical studies to human dietary and environmental

exposures. For example, *in vitro* studies with embryos indicate that both methanol and formate are embryo-toxic, at very high concentrations. While the Panel states in several places that these formate concentrations are not likely to occur from environmental exposures, the methanol concentrations that are developmentally toxic are also not likely to occur environmentally. This should be emphasized in the final summary as it is extremely relevant to the risk assessment. In fact, the formate concentrations that affect embryos (20-40 mM) are associated with acute methanol poisoning in humans, while methanol concentrations (> 200 mM) are exceedingly rare.

- Under normal circumstances, toxicologists pay particular attention to the most sensitive species. However when physiological or metabolic differences are known to exist between the most sensitive species and humans, the most sensitive species is not the most relevant or predictive. Mice appear to be the most sensitive species to the developmental effects of methanol per se. Mouse embryos *in vitro* appear to be more sensitive than rat embryos to the same methanol concentrations, suggesting an inherent sensitivity in mice. However, mice also retain a larger portion of the inhaled methanol dose than do either rats or humans. This factor leads to much higher blood methanol levels after exposure to the same inhaled dose in mice (13-18 times that in humans and 2-3 times that in rats) (Perkins et al.(50)).
- Most of the studies cited in the report indicate that the developmental effects of high doses of methanol in rodents result from exposure to methanol, not formate (Dorman et al. (54)). If the developmental toxicity of methanol is ascribed to methanol itself, then mice would inherently be 10-20 fold more sensitive to the effects than are humans (based on the comparative blood levels produced by similar doses as discussed above). Since experimental mouse studies would therefore incorporate this 10-20 fold factor kinetically, the usual uncertainty and safety factors that are normally applied for extrapolation of data from animal-to-human are canceled out and thus, should not be used.
- Several *in vitro* studies indicate that formate or formate with acidosis can be developmentally toxic. Also, formate is known to accumulate in humans after toxicologically meaningful doses of methanol. If the developmental toxicity is related to formate accumulation in humans, then neither mice nor rats are suitable animal models, since neither species metabolize formate like humans.
- The doses that would produce marked adverse developmental effects in rats (10,000 ppm) and in mice (5,000 ppm) would be lethal to humans, because of the accumulation of formate at such levels in humans. Thus, the concern in such human exposures to methanol would be more directed at life rather than at developmental effects. These doses do not increase blood formate levels in rodents. Hence, the extrapolation of results at high doses of methanol in rodent studies is exceedingly complex, is likely not relevant to human exposures, and thus, needs to be carefully considered by the Panel.
- Mice have markedly higher blood and tissue folate concentrations than those in either rats or humans. Hence, one cannot assess the role of formate in mice, since they are very metabolically different from humans. The more appropriate animal model would be another primate such as the monkey. In primate studies

of the developmental effects of methanol, using levels of methanol that exceed the usual dietary ingestion or environmental exposures (Burbacher et al. (93) and Reynolds et al. (97)), marked toxicity of methanol is absent. Subtle changes are seen in some of the parameters measured the Burbacher study, but the majority of measures were not changed. There were no effects observed in the Reynolds study at aspartame doses equivalent to 250 mg/kg doses of methanol.

- Environmental levels of methanol (200 ppm) produce blood methanol levels in a range that is obtainable from dietary exposures alone (consumption of fruits, juices, etc.). In the several human studies (Lee et al. (23), Cook et al. (21), Osterloh et al. (28)), 200 ppm exposure increased blood methanol from 1.8 mg/L to 7-8 mg/L. In the study of Leon et al. (see below), blood methanol levels as high as 3 mg/L were recorded in a placebo group of humans over a 24 week period. In none of these studies was there any formate accumulation in the blood.

Summary

- Methanol exposures in high dose aspartame studies in humans demonstrate measurable methanol levels but no formate accumulation. Thus, exposure to humans at environmental levels of methanol will not produce formate accumulation.

- Different species respond differently to methanol metabolically and toxicologically. Significant differences in mice question the use of mice as a "most sensitive" species model for human exposures.

- Adverse developmental effects of methanol in the absence of formate accumulation in animal studies are not likely relevant to humans because equivalent doses of methanol to humans would be lethal.

- Studies of developmental effects of methanol exposure in primates appear to indicate a lack of effects of methanol, even at levels well above environmental or dietary exposures.

New References (not included in draft report)

Leon AS, Hunninghake DB, Bell C, Rassin DK & Tephly TR. Safety of long-term large doses of aspartame. Arch Intern Med 149: 2318-24, 1989.